The Oxidation of Lactose, Glucose, and Galactose by Means of Neutral and Alkaline Potassium Permanganate

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DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of the Ohio State University.

By

CALVIN ADAM BUEHLER, B. Ch. E., M. Sc.

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INTRODUCTION

THE oxidation of organic compounds has for a long time been used as a method of identification for these compounds. One of the first cases of oxidation recorded is that of Scheele,[1] who in 1776 obtained oxalic acid by the oxidation of sugar with nitric acid. Later Dobereiner[1] in 1822 prepared formic acid by the oxidation of tartaric acid, and acetic acid by the oxidation of ethyl alcohol with platinum black.

During the nineteenth century quite a few classes of organic compounds were submitted to oxidation qualitatively using various oxidizing agents in acid, alkaline, and neutral media as well as different temperature conditions. The result of this lack of uniformity was a series of apparently conflicting results. On this account the work done in recent years in this laboratory, on the oxidation of organic compounds with potassium permanganate in varying alkali concentrations, under the direction of Dr. W. L. Evans, has been carried out under definite temperature conditions and the measurements have been made quantitatively in order that the true course of the reaction might be followed. Up to the present time the oxidation of ethyl alcohol,[2] of acetaldehyde, glycol and related compounds,[3] of acetol,[4] and acetone and isopropyl alcohol[5] has been completed. The results of these experiments throw considerable light on the mechanism of the reactions.

The work represented by this thesis is a continuation of the above work applied to the sugar lactose and its two hydrolytic splitting products, glucose and galactose. The problem was suggested by Dr. Evans with the following purposes in view:

1. To determine the effect of alkalinity on the character and amounts of the products of oxidation.

[1] *Cohen's Organic Chemistry*, Part I, pg. 9, 1919 Edition.
[2] Day, *J. Am. Chem. Soc.* **41**, 1267 (1919).
[3] Adkins, *J. Am. Chem. Soc.* **41**, 1386 (1919).
[4] Hoover, *J. Am. Chem. Soc.* **44**, 1730 (1922).
[5] Sefton, *J. Am. Chem. Soc.* **44**, 2271 (1922).

2. To determine the effect of temperature on the character and amounts of the products of the oxidation.

3. To determine, if possible, the mechanism of the reactions when lactose, glucose, and galactose are oxidized in neutral and varying alkali concentrations.

4. To determine the relation between the character and amounts of products when glucose and galactose are oxidized under the same conditions.

5. To determine the relation between the character and amounts of products when lactose and an equivalent amount of a mixture of glucose and galactose are oxidized under the same conditions.

HISTORICAL PART

(a) Oxidation of Lactose.

The literature shows that different products are obtained when lactose is oxidized with various oxidizing agents. On distilling milk sugar with manganese dioxide and sulfuric acid, Gmelin[6] discovered formic acid in the distillate. In oxidizing lactose with potassium dichromate and dilute sulfuric acid, Guckelberger[7] obtained an aldehyde. By boiling lactose with copper sulfate and sodium hydroxide, Boedeker and Struckman[8] isolated their so-called "galactinic" and "pektolactinic" acids to which they gave the formulas, $C_{14}H_5O_9$ and $C_{16}H_8O_{12}$ respectively. Since they obtained only galactinic acid with excess copper sulfate and sodium hydroxide, they regarded pektolactinic acid as an intermediate product. Dubrunfaut,[9] M. J. Liebig,[10] and Justus Liebig[11] carried out investigations on the action of nitric acid on lactose. Dubrunfaut found tartaric acid; M. J. Liebig, mucic and oxalic acids; and Justus Liebig added carbon dioxide and saccharic acid to the above three products. Laubenheimer[12] was the first investigator to oxidize lactose with potassium permanganate. He found that oxidation was complete to carbon dioxide and water with excess permanganate but with less permanganate he obtained oxalic and a syrupy acid which he could not identify. Haberman and Honig[13] oxidized lactose in neutral and alkaline solution with cupric hydroxide and obtained carbon dioxide, formic acid, lactic acid, glycollic acid, and some non-volatile acids. It is interesting to note that they obtained the same products with galactose. Greifenhagen, König, and Scholl[14] devised a method for the determination of carbohydrates by oxidation in alkaline solution with potassium permanganate at the boiling temperature. They obtained carbon dioxide and oxalic acid as

[6] Pog. Ann. **16,** 55 (1829).
[7] Ann. **64,** 98.
[8] Ann. **100,** 264.
[9] Compt. rend. **42,** 228 (1856).
[10] Comp. rend. **49,** 341 (1859).
[11] Ann. **113,** 1.
[12] Ann. **164,** 283.
[13] Monatsh. **5,** 209 (1885).
[14] Biochem. Z. **35,** 169 (1911).

products of the reaction with lactose, and have proposed the following equation to represent the reaction which takes place:

$$C_{12}H_{22}O_{11} + 10\ O_2 \rightarrow 4\ H_2C_2O_4 + 4\ CO_2 + 7H_2O$$

(b) Hydrolytic Splitting of Lactose in Acid and Alkaline Solution.

Fudakowski[15] first called attention to the fact that lactose splits into two "C_6" containing sugars when treated with dilute sulfuric acid. One year later he[16] proved that the two sugars resulting from the acid hydrolysis of lactose were glucose and galactose.

The literature shows that a greater variety of products are formed when lactose is broken up in alkaline solution. On boiling lactose with sodium hydroxide, Hoppe-Seyler[17] obtained lactic acid (10-20% of the weight of sugar), formic acid, and pyrocatechol. Nencki and Sieber[18] also obtained lactic acid using potassium hydroxide. Duclaux[19] observed that lactose in alkaline solution in the sunlight formed carbon dioxide, lactic acid (about 50% of the weight of sugar), acetic acid, formic acid, and alcohol. Since lactic acid with potassium hydroxide produced alcohol in the sunlight, Duclaux thought that the alcohol was formed through the lactic acid as an intermediate product.

The action of alkalies on lactose[20] gives similar products to those obtained in the case of glucose and galactose. Therefore lactose may hydrolyze in the presence of alkalies, as well as in the presence of acids, into glucose and galactose. Lobry de Bruyn called attention to this possibility.[21] Later he[22] succeeded in partially verifying this idea when he isolated galactose from a solution of lactose which had been treated with potassium hydroxide. Unfortunately he was unable to get a test for glucose in this reaction mixture.

(c) Oxidation of Glucose.

The literature shows that more work has been done on the oxidation of glucose and sucrose than with any of the other sugars.

[15] Ber. **8,** 599 (1875).
[16] Ber. **9,** 42 (1876).
[17] Ber. **4,** 346 (1871).
[18] J. prakt. *Chem. N. F.* **24,** 502 (1882).
[19] Chem. Centr. 1894 (1) 169.
[20] Hoppe-Seyler, Ber. **4,** 346 (1871).
Nencki & Sieber, *J. prakt. Chem. N. F.* **24,** 502.
Duclaux, Chem Centr. 1894 (1) 169.
Meisenheimer, Ber. **41,** 1009 (1908).
[21] Rec. trav. chim. **15,** 96 (1896).
[22] Rec. trav. chim. **18,** 147 (1899).

The products from glucose, as in the case with lactose, are varied depending on the oxidizing potential of the oxidizing agent, the temperature, and other experimental conditions.

In boiling glucose with manganese dioxide and dilute sulfuric acid, Hünefeld[23] obtained carbon dioxide, formic acid, and acetic acid. By boiling with lead peroxide in water, Stürenburg[24] decomposed the sugar completely to carbon dioxide and formic acid. With manganese dioxide and sulfuric acid, Liebig[25] succeeded in getting an aldehyde and acrolein (?). Gorup[26] also obtained complete oxidation to carbon dioxide and formic acid using ozone in the presence of soda. Reichardt[27] and Felsko[28] obtained a gummy mass from which they isolated their so-called "gummisäure" by the action of alkaline cupric hydroxide on glucose. Claus[29], using the same oxidizing agent as Reichardt, proved the presence of carbon dioxide, tartronic acid, formic acid, and oxalic acid in the reaction mixture. He thought that acetic acid was also present and later[30] advanced evidence showing that Reichardt and Felsko's "gummisäure" was a mixture of acids consisting largely of tartronic acid.

Kiliani [31] oxidized an aqueous solution of dextrose with silver oxide at ordinary temperature to carbon dioxide, oxalic acid, and glycollic acid. At the boiling temperature the amount of glycollic acid decreased to a very small amount. With an alkaline copper solution Maumene[32] formed his so-called "hexepinsäure" and "triejinsäure" as well as gluconic and the lower acids to carbonic together with much lactic acid. On boiling glucose with water and cupric hydroxide, Haberman and Hönig[33] obtained carbon dioxide, glycollic acid, glyceric acid (?) and erythroglyceric acid (?). No tartronic acid was present. On making the mixture alkaline with barium oxide, they obtained gluconic acid in addition to the above products.

[23] *J. prakt. Chem.* **7**, 44 (1836).
[24] Ann. **29**, 291.
[25] Ann. **113**, 16.
[26] Ann. **125**, 211.
[27] Ann. **127**, 297.
[28] Ann. **149**, 356.
[29] Ann. **147**, 114.
[30] *J. prakt. Chem.* N. F. **4**, 63 (1871).
[31] Ann. **205**, 191.
[32] J. fabr. **27**, 29; through *Lippmann's Chem. der Zuckerarten* Vol. I, pg. 310.
[33] Monatsh. **3**, 651 (1883).

Herzfeld[34] conducted chlorine into an aqueous solution of glucose after which he added silver oxide. Gluconic and saccharic acids resulted as products of this oxidation. Later Herzfeld[35] again obtained gluconic acid and in addition formic and glycollic acids when he boiled glucose with mercuric oxide and barium hydroxide. Tollens[36] oxidized glucose with silver oxide and obtained glycollic acid. On using an ammoniacal silver solution treated with sodium hydroxide, the reaction proceeded as follows:

$$C_6H_{12}O_6 + 6\ Ag_2O \rightarrow 6HCOOH + 12\ Ag$$

In addition a small amount of oxalic acid and probably traces of carbon dioxide were also formed.

On exposing an alkaline solution of glucose to the air and sun, Duclaux[37] succeeded in decomposing the sugar into alcohol (as high as 5% of the weight of sugar), carbon dioxide, formic acid, acetic acid, and oxalic acid. Using mercuric oxide alone as the oxidizing agent, Heffter[38] obtained good yields of gluconic acid. With the same oxidizing agent as Herzfeld's (mercuric oxide with barium hydroxide), Bruhns [39] obtained, in addition to Herzfeld's products, trihydroxybutyric acid. Smolka[40] decomposed glucose completely to carbon dioxide and water with excess potassium permanganate at the boiling temperature. With less potassium permanganate at ordinary temperature there resulted formic and oxalic acids.

Allein and F. Gaud[41] obtained dihydroxyphenylpropionic, saccharic, and tartronic acids, as the decomposition products of glucose in alkaline copper solution. They advanced the following equations to account for the above decomposition products:

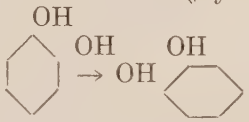
[34] Ann. **220,** 358.
[35] Ann. **245,** 32.
[36] Ber. **16,** 921 (1883).
[37] Compt. rend. **103,** 881 (1885)
[38] Ber. **22,** 1049 (1889).
[39] *Zeit. Ver Zuckerind.* **36,** 110; through *Lippmann's Chem. der Zuckerarten,* Vol. I, pg. 310.
[40] Montash. **8,** 26 (1888).
[41] Chem. Centr. 1894, (2) 776.

$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$ (Lactic acid)

$CH_3CHOHCOOH + O_2 \rightarrow COOHCHOHCOOH + H_2O$
(Tartronic acid)

$COOHCHOHCOOH + CO_2 + H_2O \rightarrow COOHC(OH)_2.C(OH)_2COOH$
(Dihydroxytartaric acid)

$COOHC(OH)_2.C(OH)_2COOH + 2CO_2 \rightarrow$ $+ O_{10}$
(Pyrocatechol)

$CH_3CHOHCOOH +$  $CH_2.CH.COOH$
(Dihydroxyphenylpropionic acid)

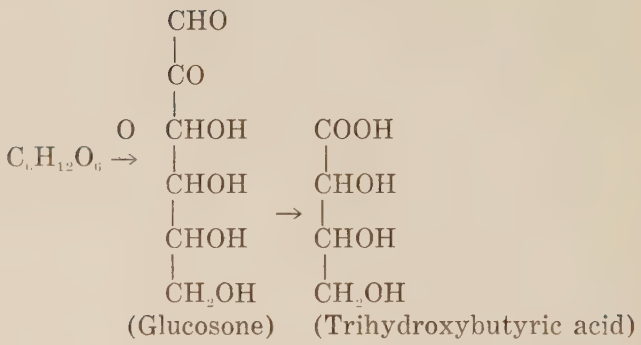
Kjeldahl[42] boiled 1 mol glucose with Fehlings solution and obtained 2.6 mols acid. Carbon dioxide and mesoxalic acid were identified as products of the reaction and the remainder of the acidity was attributed to formic, glycollic, glyceric, trihydroxybutyric, arabonic, and gluconic acids. Perdrix[43] found that glucose oxidized as follows with potassium permanganate in acid solution:

$$\begin{array}{lll} CH_2OH & + 2O \rightarrow 0.5\ CO_2 + 0.5\ HCOOH + H_2O \\ | & \\ (CHOH)_4 & + 4O \rightarrow \qquad\qquad 4.0\ HCOOH \\ | & \\ CHO & + 1.50 \rightarrow 1.0\ CO_2 \qquad\qquad + 0.5\ H_2O \end{array}$$

$$C_6H_{12}O_6 + 7.5\ O \rightarrow 1.5\ CO_2 + 4.5\ HCOOH + 1.5\ H_2O$$

Using hydrogen peroxide in the presence of small amounts of iron, Cross, Bevan, and Smith[44] oxidized dextrose to formic acid (15-20%), acetic acid (4-7%), and non-volatile acids including tartronic acid (19-27%). Morrell and Crofts[45] obtained glucosone by the action of hydrogen peroxide in the presence of ferrous sulfate on glucose. Greater amounts of hydrogen peroxide produced glycollic, glyoxylic, oxalic, and trihydroxybutyric acids. The authors explained the formation of trihydroxybutyric acid as follows:

[42] Chem. Zeit. **19**, (2) 218 (1895).
[43] Compt. rend. **123**, 945 (1896).
[44] Chem. Centr. 1898 (2) 19
[45] *J. Chem. Soc.* T **75**, 787 (1899); T **77**, 1221 (1900) T **81**, 666 (1902); T **83**, 1284 (1903).



They thought the formation of the other acids was not explicable on the intermediate formation of glucosone.

Tarugi and Nicchiotti[46] found that potassium ferricyanide in the presence of alkali reacted on glucose (all three being in normal solution) with the formation of carbon dioxide and acetic acid. They proposed the following reaction as an explanation for these facts (?):

$$10\ K_3Fe(CN)_6 + 17KOH + 2C_6H_{12}O_6 \rightarrow$$
$$12\ H\ O + 10\ K_4Fe(CN)_6 + 2CH_3COOK + K_2CO_3$$

Tenth-normal solution produced gluconic and acetic acids or, in addition to these, carbon dioxide, depending on the quantity of alkali. Fortieth-normal solutions produced practically d-gluconic acid alone.

McLeod[47] heated 100 grams d-glucose on the water bath for 8 hours with an excess of 10 mols copper acetate and obtained 12.2 grams carbon dioxide, 10.03 grams formic acid, 8.9 grams anhydrous oxalic acid, and 3.25 grams glycollic acid. Nef[48] treated 114 grams glucose with cupric hydroxide and sodium hydroxide and obtained 3.83 grams carbon dioxide, 14.71 grams formic acid, and 99.8 grams non-volatile acids, which contained 22 grams glycollic acid, 14 grams glyceric acid, 30 grams trihydroxybutyric acid, and 30 grams hexonic acids. On oxidizing 10 grams dextrose with silver oxide made alkaline with barium hydroxide, he[49] ob-

[46] Gazz. chim. ital. **27,** 131; through *Lippmann's Chem. der Zuckerarten,* Vol. I, pg. 308.
[47] *Am. Chem. J.* **37,** 20 (1907).
[48] Ann. **357,** 277.
[49] Ann. **357,** 289.

tained 2.256 grams carbon dioxide, 8.534 grams anhydrous oxalic acid, and 3.845 grams formic acid.

Using hydrogen peroxide in potassium hydroxide solution as an oxidizing agent on 2 grams glucose, Spoehr[50] recovered 2.0 grams formic acid, 0.163 grams carbon dioxide, glycollic acid, and α hydroxymethyl d-arabonic acid. With an excess of hydrogen peroxide in the presence of ferric sulfate 2 grams d-glucose decomposed into 2.08 grams carbon dioxide, 0.092 grams formic acid, and 0.70 grams oxalic acid. Glattfeld[51] repeated Spoehr's work and found the latter's α hydroxymethyl d-arabonic acid to be d-arabonic acid and in addition proved that d-erythronic, l-threonic, and dl-glyceric acids were present. Besides, Glattfeld oxidized glucose in alkaline solution with air and also found formic acid, γ d-arabonic lactone, d-erythronic lactone, and dl-glyceric acid.

Greifenhagen, König and Scholl[52] obtained only carbon dioxide and oxalic acid from glucose, in their method for the determination of carbohydrates by oxidation with alkaline potassium permanganate. Their equation for the reaction is as follows:

$$C_6H_{12}O_6 + 5\ O_2 \rightarrow 2H_2C_2O_4 + 2\ CO_2 + 4\ H_2O$$

On distilling a solution containing glucose and hydrogen peroxide, Effront [53] succeeded in getting 1-9% alcohol, 60% volatile acids (2 parts acetic acid to 8 parts formic acid), and oxalic acid. The amount of the latter depended on the length of distillation and disappeared entirely after complete transformation of the sugar. Witzemann[54] oxidized glucose with potassium permanganate in the presence of varying amounts of potassium hydroxide and obtained carbon dioxide and oxalic acid as the oxidation products. The yields of carbon dioxide and oxalic acid were 100 and 0% respectively from neutral up to 0.0280N alkali. At this point the formation of oxalic acid began and reached a maximum of 42% in 1.82N potassium hydroxide.

[50] *Am. Chem. J.* **43**, 227 (1910).
[51] *Am. Chem. J.* **50**, 135 (1913).
[52] loc. cit.
[53] Compt. rend. **154**, 1296 (1912).
[54] *J. Am. Chem.* Soc. **38**, 150 (1916).

(d) Oxidation of Galactose.

The literature contains just a few references to the oxidation of galactose and the greater part of that work has been accomplished in recent years. Haberman and Hönig[55] oxidized galactose with cupric hydroxide in neutral and alkaline solutions and in both cases obtained carbon dioxide, formic acid, lactic acid, and glycollic acid, together with some non-volatile acids. Morrell and Crofts[56] treated galactose with hydrogen peroxide in the presence of ferrous sulfate with the hope of obtaining galactosone, since they succeeded in getting glucosone by treating glucose in a similar manner. However, they were not certain that they actually obtained galactosone.

McLeod[57] heated 100 grams d-galactose at the boiling point with an excess of 10 mols of aqueous copper acetate and obtained 4.03 grams carbon dioxide, 11 grams formic acid, and 12.3 grams anhydrous oxalic acid. On oxidizing 10 grams d-galactose with silver oxide made alkaline with barium hydroxide, Nef[58] succeeded in getting 2.995 grams carbon dioxide, 6.447 grams oxalic acid, and 4.3625 grams formic acid. Anderson[59] decomposed 118 grams d-galactose in an alkaline solution of cupric hydroxide and formed 2.49 grams carbon dioxide, 15.69 grams formic acid, and 102.1 grams non-volatile hydroxyacids from which he isolated 13 grams d-galactonic acid, 5.33 grams d-talonic acid, 0.5 grams oxalic acid, 2.58 grams l-threonic lactone, traces of d-erythronic lactone, about 11 grams dl-glyceric acid, and 11.75 grams glycollic acid.

Spoehr[60] oxidized 2 grams d-galactose with alkaline hydrogen peroxide and obtained 0.143 grams carbon dioxide, 2.46 grams formic acid, glycollic acid, and α hydroxymethyl-d-lyxonic acid. Using an excess of hydrogen peroxide in the presence of iron salts, Spoehr obtained from 2 grams d-galactose, 2.03 grams carbon dioxide, 0.092 grams formic acid, and 1.13 grams oxalic acid. Nef[61]

[55] loc. cit.
[56] *J. Chem Soc.* T **75**, 787.
[57] loc. cit.
[58] Ann. **357**, 289.
[59] *Am. Chem. J.* **42**, 401 (1909).
[60] loc. cit.
[61] Ann. **403**, 204.

passed air through an alkaline solution of d-galactose and recorded as oxidation products: 2.55 grams carbon dioxide, 25.62 grams formic acid, glycollic acid, traces of oxalic acid, and dl-glyceric acid together with l-threonic lactone, a little d-erythronic acid γ lactone, d-lyxonic acid γ lactone, and some l-xylonic acid γ lactone. On repeating Anderson's work he obtained practically the same results except that Anderson's α hydroxymethyl-d-arabonic acid and α hydroxymethyl-d-lyxonic acids were shown to be d-arabonic and d-lyxonic acids respectively. With d-galactose in alkaline hydrogen peroxide he succeeded in getting large amounts of formic acid, traces of oxalic acid, glycollic, dl-glyceric, l-threonic, d-erythronic, d-lyxonic, l-xylonic, d-galactonic, and d-talonic acids.

EXPERIMENTAL PART

(a) MATERIALS.

(1) Sugars.

All the sugars used in the oxidations were the C. P. special products from the Specials Chemical Company, Highland Park, Illinois. The analysis on the three sugars as furnished by them is as follows:

Lactose:

Specific rotation $(\alpha)_D^{20}$	+52.5 (2.000 grms. per 100cc.)
Ash	0.05%
*Moisture	0.15%

No sulfates, chlorides, Al, Ca or heavy metals. Free of glucose and other sugars.

*The sample was originally thought to be anhydrous but by heating at 50 degrees C. for one hour and then at 90 degrees C. for fifteen hours,[83] the loss of water amounted to 5.15%, hence the sample must have contained one molecule of water.

d-Glucose (anhydrous):

Specific rotation $(\alpha)_D^{20}$	+52.5 (9.097 grms. per 100cc.)
Ash	0.05%
Moisture	0.10%

No sulfates, chlorides, Al, Ca, or heavy metals. Free from dextrin, maltose, etc.

d-Galactose:

Specific rotation $(\alpha)_D^{20}$	+80 (10.000 grms. per 100cc.)
Ash	0.05%
Moisture	0.10%

No sulfates, chlorides, Al, Ca, or heavy metals. Free from glucose.

[83] *J. Ind. & Eng. Chem.* **13**, 688 (1921).

(2) Carbon Dioxide—Free Water.

Water free from carbon dioxide was prepared by bubbling carbon dioxide—free air through distilled water over night. One drop of 0.0523N potassium hydroxide gave a decided pink color to about 300 cc. of this water to which one drop of phenolphthalein had been added.

(3) Potassium Permanganate.

Baker and Adamson's C. P. potassium permanganate was used, the oxidizing power of which was found to be 98.65% of the theoretical when weighed samples were treated with an excess of pure oxalic acid ($(COOH)_2 \cdot 2\ H_2O$) and titrated back with standard potassium permanganate solution. The standard potassium permanganate solution (about one-tenth normal), which was used in the determination of oxalic acid, was standardized about once each month against Bureau of Standards sodium oxalate.

(4) Potassium Hydroxide.

J. T. Baker's C. P. potassium hydroxide purified by alcohol was used. A stock solution of this potassium hydroxide of about two normal strength, standardized against 0.2515N hydrochloric acid using methyl orange as indicator, was used in the oxidations. The carbon dioxide content of this solution was determined by the method given below. Another potassium hydroxide solution of about one-twentieth normal strength, previously freed from carbon dioxide by the addition of a small amount of barium hydroxide, was standardized against pure potassium acid phthalate[64] using phenolphthalein as the indicator.

(5) Phosphoric Acid.

The phosphoric acid used in the acetic acid distillations was Mallinckrodt's 85%. Blank analyses on 20 cc. of this acid gave volatile acids equivalent to 0.25 cc. of 0.0523N potassium hydroxide.

(b) APPARATUS AND MANIPULATION.

Each oxidation was carried out as follows:

Thirteen grams of potassium permanganate were placed in a

[64] *J. Ind. & Eng. Chem.* 7, 29 (1915).

two liter, round-bottom flask. A definite amount of the standard potassium hydroxide solution was then pipetted out of the stock solution into a 500 cc. volumetric flask which was next filled up to the mark with carbon dioxide-free water. However, for alkali concentrations below one gram potassium hydroxide in 500 cc., 25 cc. of the stock solution was diluted up to 250 cc. and the proper amount of this dilute solution was then used. The alkaline solution so prepared was placed in the round-bottom flask into which the potassium permanganate had already been introduced. The flask was then closed by a rubber stopper, fitted with a four-inch mercury-sealed stirrer, A, and the glass tubes, B, C, and D (See Plate I). The sugar solution was introduced through tube, B, by means of a burette connected by rubber tubing. Tube C was used to draw up the oxidation mixture in order to determine when the potassium permanganate had been completely decolorized. It served also in the case of low alkalinities as an entrance for carbon dioxide-free air. Tube D was used only in the low alkalinity experiments for removing the carbon dioxide in the space above the oxidation mixture by absorption in an attached Geissler bulb. After being closed the flask was clamped into position in the thermostat, and agitation through a shaft driven by a one-sixth horse power motor was begun.

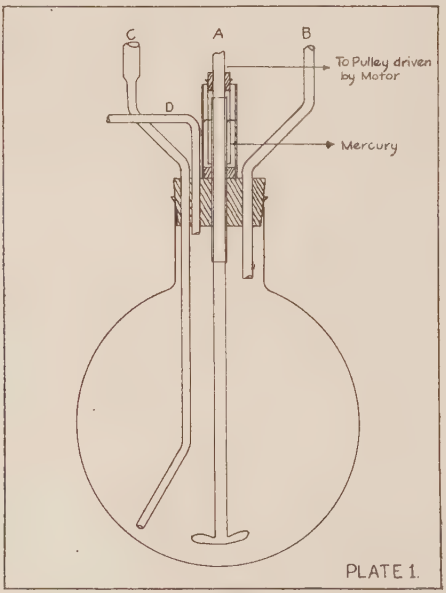


PLATE 1.

Water was used in the thermostat at all temperatures, although at 50° and 75° it was found advisable to cover the water with a layer of paraffin. The bath was heated by two electric hot plates at the two higher temperatures, the current to one of the hot plates being automatically controlled by means of a mercury-toluene regulator and a lamp bank. At 25 , only one hot plate was used, and it was found necessary, since the oxidations at this temperature were carried out during the summer months, to introduce a cooling coil through which water, cooled by an ice bath, flowed. The temperature of the bath was constant to within 0.25 of 1°.

The sugar solution was made up in such a way that each 25 cc. of solution contained 1.80126 grams sugar. As soon as the temperature of the bath was regulated at the desired temperature, a 50 cc. burette was filled up to the 25 cc. mark with the sugar solution and attached to the proper tube leading to the flask. The glass tube, C (Plate I), was closed with a rubber stopper and for alkali concentrations below 1 gram in 500 cc., a calcium chloride tube and a Geissler bulb, in series for carbon dioxide absorption, were connected to the tube D. Due to the fact that some of the runs at neutral and the lower alkalinities were 10 days or more in length, it was found necessary to attach a small calcium chloride tube, which was weighed with the Geissler bulb, directly in front of it in order to prevent any loss in weight of the potassium hydroxide solution due to absorption of its vapor by the main calcium chloride tube. As this absorption train was not needed with the higher alkalinities, in those cases the tube D was closed.

When the contents of the flask had attained the temperature of the bath, the sugar solution was introduced from the burette at the rate of 0.5 cc. every 5 minutes until 25 cc. had been added. This slow rate of addition was necessary because of the intense local heating occasioned by the interaction of the sugar and the oxidizing agent at the higher alkalinities. After the sugar solution had been added in the desired amount the agitation was continued from 12 hours to 10 days or more, depending on the temperature and strength of alkali used in the oxidation. As soon as the agitation had continued for the proper length of time, the permanganate in excess was removed by decomposing it with a small excess of sodium hypophosphite. For the higher alkalinities the oxidation was now finished and the flask was removed from the bath. For those containing less than 1 gram of potassium hydroxide per liter.

25 cc. of the stock solution was added after the permanganate had been decolorized. The carbon dioxide in the air above the oxidation mixture was then removed by attaching an aspirator to the Geissler bulb and aspirating 3 to 4 liters of carbon dioxide—free air through the flask. The flask was then removed from the bath.

For filtering the apparatus devised by Evans and Day[65] was used. The filtrate was made up to one or two liters depending on the alkalinity, and samples of from 50 to 200 cc. were used to make the various analyses.

(c) METHODS OF ANALYSIS.

The only products formed in these oxidations were carbon dioxide, oxalic acid, and acetic acid, the quantitative determinations of which are discussed below.

(1) Carbon Dioxide.

The determinations for carbon dioxide on the solutions were made by Foulk's method.[66] One or two hundred cubic centimeters of the solution, depending on the alkalinity, were treated with an excess of hydrochloric acid; and the carbon dioxide, after being conducted through a condenser, sulfuric acid, a sulfuric acid solution of silver sulfate, and a drying tube of calcium chloride, was absorbed in caustic potash solution in a Geissler bulb. The method of tares was used in weighing the Geissler bulbs, and a correction was applied for the potassium hydroxide solution used in the oxidations. In some of the oxidations (low alkalinities) there was considerable carbon dioxide carried over into the Geissler bulb attached to the oxidation flask. This amount was added to that found by an analysis of the solution.

(2) Oxalic Acid.

Fifty or one hundred cubic centimeters of the solution were pipetted into a 400 cc. beaker after which about 100 cc. of distilled water were added. The solution so obtained was then heated on the hot plate to near boiling after which 10 cc. of 50% acetic acid solution were added. With the use of phenolphthalein this amount of acid was found to be sufficient to acidify the solution in all cases.

[65] *J. Am. Chem. Soc.* **41**, 1272 (1919).
[66] Foulk's "*Notes on Quantative Analysis*" 1914 Edition, pg. 222, McGraw-Hill Book Co.

Twenty cubic centimeters of 5% calcium acetate solution were then introduced and the mixture was boiled for a short time after which it was allowed to stand for several hours on the hot plate, which remained warm for some time after the gas was turned off. The precipitate so obtained, which was of a good physical character, was filtered and washed several times with hot water on purified asbestos in a Gooch crucible. It was then transferred together with the asbestos to the same 400 cc. beaker which originally contained it, after which about 200 cc. of distilled water were added. This mixture was then acidified with 20 cc. 1 : 1 sulfuric acid, heated to 70°-80° and titrated with standard potassium permanganate solution in the usual manner.

(3) Acetic Acid.

The method of Stillwell and Gladding[67] as modified by Adkins[68] was used for the determination of acetic acid. By this method 100 cc. of the solution were placed in a 500 cc. round-bottom flask together with a few pieces of porous plate to prevent bumping. The flask was fitted with a two-hole rubber stopper supplied with a cylindrical dropping funnel and a Kjeldahl bulb. The Kjeldahl bulb was in turn connected to a long Liebig condenser which emptied through an adaptor into a cylindrical dropping funnel graduated in 25 cc. divisions. The dropping funnel was connected to a 750 cc. Erlenmeyer flask by means of a two-hole rubber stopper. After the apparatus had been set up, 20 cc. of syrupy phosphoric acid were added (this amount was found to be a considerable excess over that required to neutralize the alkali present in all cases), and the distillation was begun. After 50 cc. of the distillate had collected 25 cc. of hot water were added to the contents of the distilling flask and the distillation was continued until 25 cc. more had collected, when 25 cc. of hot water were again added and so on until 400 cc. of distillate had collected. In this manner the volume in the distillation flask was kept below 125 cc. in all cases and in most cases below 95 cc. The distillation was carried out at a no greater rate than 100 cc. distillate per 30 minutes in order to prevent the carrying over of some phosphoric acid. All the acetic acid was found by experiment to have been carried over by this amount of distillate. The distillate was freed from carbon dioxide by bubbling carbon dioxide—free air through it for 10-15 minutes, after which

[67] *J. Am. Chem. Soc.* **4**, 105 (1882).
[68] *J. Am. Chem. Soc.* **41**, 1388 (1919).

it was titrated with a 0.0523N potassium hydroxide solution using phenolphthalein as an indicator. The alkali used after deducting 0.25 cc. for the blank on 20 cc. phosphoric acid was calculated to acetic acid.

(d) EXPERIMENTAL DIFFICULTIES.

(1) Acetic Acid.

The method of analysis for acetic acid presented some difficulty due to the fact that this acid was present in the oxidation mixture in such small quantities. In no case was the amount of acetic acid obtained greater than 0.055 grams (See Experiment 73, Table, XII) which amounts to 3% of the carbon return. This means that the actual determination for acetic acid was made on a sample containing 0.0055 grams or equivalent to 0.3% of the carbon return, since one-tenth of the oxidation filtrate was used for this analysis. In order to obtain concordant results with such small amounts of acetic acid, the distillations must be carried out as near as possible at the same rate (not faster than 50 cc. in 30 minutes) and great pains must be taken to see that the carbon dioxide is completely removed by bubbling carbon dioxide-free air through the distillate for about 15 minutes.

The identification of the volatile acid, driven over in the distillation, as acetic acid could not be accomplished since the amounts present were not sufficient to cause the separation of silver acetate, and the subsequent analysis of the purified silver salt. However, since formic acid was shown to be absent in the distillate by the method of Jones,[69] it was assumed that the volatile acid was acetic acid. Further evidence in favor of this assumption was collected when positive results were obtained according to Arnold's[70] method for the detection of acetic acid which was carried out as follows:

Two cubic centimeters of a solution of 1 gram para-amino-acetophenone dissolved in 80-100 cc. of water with the addition of hydrochloric acid and 1 cc. of a solution of 1% sodium nitrite were mixed in a graduated cylinder. On standing a short time a yellow precipitate separated out. To this mixture 3 cc. of the solution to be treated were added and then 1 to 2 drops of ammonium hydroxide. On shaking, this mixture turned to a deep red color and the precipitate was still present.

[69] *Am. Chem. J.* **17**, 539 (1895).
[70] Chem. Centr. 1899 ii 146.

Fifteen to twenty drops of the above mixture were placed in a test tube and 15-20 cc of concentrated hydrochloric acid were added. If acetic acid was present, the solution turned a purple violet color.

The positive results by the above method were obtained from the phosphoric acid distillate from several oxidation solutions which had been concentrated to a small volume. Very decided results were obtained from the alkaline oxidation solution previous to distillation with phosphoric acid but it was found that a blank containing approximately the same amounts of reagents gave similar results. Therefore, this test cannot be used satisfactorily in the presence of alkali, oxalic acid, and the other products present in the oxidation solution.

(2) Completeness of Oxidations.

The difficulty in this work, which required the greatest amount of time to overcome, was the failure of the oxidations at neutral and the lower alkalinities to go to completion. This was particularly true in the oxidations at neutral and lower alkalinities at 25° where after twenty days agitation, the carbon yields were still too low to be acceptable. Unfortunately there is no way of telling when the reaction is completed except by stopping the oxidations and analyzing the filtered solutions. In case they were not complete, another run was started and so forth until satisfactory yields were obtained. In the case of lactose at the higher alkalinities at 25°, the oxidations were repeated three times when finally after five days agitation very satisfactory results were obtained.

However this failure of the reaction to go to completion at neutral in the same length of time as at the higher alkalinities led to the discovery of oxalic acid as an intermediate product in the neutral and low alkalinity oxidations. The first runs at neutral contained an acid, which formed an insoluble calcium salt, although duplicate runs did not give the same amounts of this acid nor was the carbon return satisfactory in either case. After repeating these oxidations at neutral, this time continuing the agitation for three days, no acid, which formed an insoluble calcium salt, was present in the oxidation solutions, and the analyses on duplicate runs checked each other. That this acid was oxalic acid is shown below under the discussion of oxalic acid.

(e) DATA AND CURVES.

The results of the analyses of the different oxidation mixtures are shown in the Tables I-XII as well as by the curves on Figs. 1-6. I have taken the liberty to discard quite a few experiments, which were obviously in error due to incomplete oxidation or faulty manipulation.

TABLE I.

LACTOSE ($C_{12}H_{22}O_{11}$. H_2O) AT 25 DEGS. C.

No. of Expt.	Normality of KOH	Wt. of sugar G.	Wt. of CO_2 G.	Wt. of $(COOH)_2$ G.	Wt. of CH_3COOH G.	Carbon Yield %
1	0.1010	1.80485	0.706	1.929	0.021	99.1
2	0.2020	1.80845	0.713	1.929	0.019	99.1
3	0.4040	1.80413	0.728	1.912	0.022	99.4
4	1.0100	1.80341	0.768	1.861	0.021	99.0

TABLE II.

LACTOSE ($C_{12}H_{22}O_{11}$. H_2O) AT 50 DEGS. C.

5	0.0000	1.80413	2.500	0.000	.....	
6	0.0101	1.80485	2.491	0.000	.....	
7	0.0202	1.80845	2.260	0.283	0.032	97.4
8	0.0404	1.80845	1.469	1.101	.....	
9	0.1011	1.80845	0.728	1.945	.....	
10	0.2022	1.80413	0.729	1.892	0.021	98.5
11	0.4044	1.80341	0.746	1.846	0.022	97.8
12	1.0110	1.80341	0.788	1.778	.....	

TABLE III.

LACTOSE ($C_{12}H_{22}O_{11}$. H_2O) AT 75 DEGS. C.

13	0.0000	1.80341	2.571	0.000	0.030	98.9
14	0.0202	1.80485	2.597	0.000	0.033	100.0
15	0.0403	1.80845	2.102	0.505	0.029	99.5
16	0.0807	1.80485	0.915	1.780	0.030	102.0
17	0.1008	1.80485	0.801	1.891	0.027	101.6
18	0.2016	1.80845	0.816	1.878	0.024	101.4
19	0.4033	1.80413	0.838	1.827	0.031	100.9
20	1.0082	1.80341	0.885	1.750	0.034	100.1

TABLE IV.

MIXTURE d-GLUCOSE AND d-GALACTOSE AT 25 DEGS. C.

21	0.1010	1.80485	0.512	2.149	0.016	99.6
22	0.2020	1.80845	0.554	2.096	0.016	99.1
23	0.4040	1.80413	0.617	2.055	0.019	100.3
24	1.0100	1.80485	0.701	1.967	0.021	100.3

TABLE V.

MIXTURE d-GLUCOSE AND d-GALACTOSE AT 50 DEGS. C.

No. of Expt.	Normality of KOH	Wt. of sugar G.	Wt. of CO_2 G.	Wt. of $(COOH)_2$ G.	Wt. of CH_3COOH G.	Carbon Yield %
25	0.0000	1.80413	2.562	0.000	0.027	98.4
26	0.0101	1.80485	2.564	0.000	.....	
27	0.0202	1.80845	2.503	0.115	.....	
28	0.0404	1.80485	1.446	1.143	.....	
29	0.1011	1.80845	0.614	2.076	0.024	101.0
30	0.2022	1.80413	0.646	2.038	0.021	100.9
31	0.4044	1.80341	0.680	1.954	.....	
32	1.0110	1.80845	0.768	1.831	0.022	97.7

TABLE VI.

MIXTURE d-GLUCOSE AND d-GALACTOSE AT 75 DEGS. C.

33	0.0000	1.80341	2.581	0.000	0.038	99.8
34	0.0202	1.80413	2.594	0.000	0.037	100.1
35	0.0403	1.80845	2.577	0.000	0.030	98.9
36	0.0807	1.80485	1.272	1.351	0.031	99.7
37	0.1008	1.80485	0.822	1.871	0.033	102.0
38	0.2016	1.80845	0.836	1.861	0.030	101.8
39	0.4033	1.80413	0.834	1.851	0.032	101.7
40	1.0082	1.80341	0.889	1.757	0.032	100.4

TABLE VII.

d-GLUCOSE ($C_6H_{12}O_6$) AT 25 DEGS. C.

41	0.1010	1.80485	0.586	2.048	0.021	99.0
42	0.2020	1.80845	0.622	2.041	0.019	99.8
43	0.4040	1.80413	0.648	1.982	0.025	99.2
44	1.0100	1.80341	0.715	1.881	0.020	97.7

TABLE VIII.

d-GLUCOSE ($C_6H_{12}O_6$) AT 50 DEGS. C.

45	0.0000	1.80413	2.550	0.000	0.024	97.8
46	0.0101	1.80485	2.597	0.000	.....	
47	0.0202	1.80845	2.550	0.055	0.016	99.1
48	0.0404	1.80413	1.427	1.155	0.024	98.0
49	0.0808	1.80413	0.716	1.984	.....	
50	0.1617	1.80341	0.727	1.949	.....	
51	0.4044	1.80341	0.748	1.868	.....	
52	1.0110	1.80341	0.779	1.771	.....	

TABLE IX.

d-GLUCOSE ($C_6H_{12}O_6$) AT 75 DEGS. C.

No. of Expt.	Normality of KOH	Wt. of sugar G.	Wt. of CO_2 G.	Wt. of $(COOH)_2$ G.	Wt. of CH_3COOH G.	Carbon Yield %
53	0.0000	1.80341	2.559	0.000	0.028	98.4
54	0.0101	1.80413	2.594	0.000	0.022	99.4
55	0.0202	1.80845	2.598	0.000	0.027	99.5
56	0.0403	1.80485	2.611	0.000	0.028	100.3
57	0.1008	1.80485	0.907	1.785	0.024	101.5
58	0.2016	1.80845	0.822	1.863	0.032	101.5
59	0.4033	1.80413	0.842	1.832	0.028	101.1
60	1.0082	1.80341	0.894	1.717	0.028	99.0

TABLE X.

d-GALACTOSE ($C_6H_{12}O_6$) AT 25 DEGS. C.

61	0.1010	1.80341	0.458	2.210	0.018	100.0
62	0.2020	1.80845	0.538	2.143	0.026	100.7
63	0.4040	1.80413	0.574	2.099	0.021	100.4
64	1.0100	1.80341	0.656	1.984	0.022	99.4

TABLE XI.

d-GALACTOSE ($C_6H_{12}O_6$) AT 50 DEGS. C.

65	0.0000	1.80341	2.536	0.000	0.033	97.8
66	0.0101	1.80485	2.566	0.000	0.036	99.1
67	0.0202	1.80845	2.575	0.000	.....	
68	0.0404	1.80485	1.533	1.043	.....	
69	0.0808	1.80845	0.611	2.052	.....	
70	0.1617	1.80845	0.592	2.079	.....	
71	0.4044	1.80845	0.645	2.009	.....	
72	1.0110	1.80413	0.758	1.861	0.022	98.7

TABLE XII.

d-GALACTOSE ($C_6H_{12}O_6$) AT 75 DEGS. C.

73	0.0000	1.80341	2.551	0.000	0.055	99.6
74	0.0101	1.80413	2.571	0.000	0.049	99.9
75	0.0202	1.80845	2.568	0.000	0.050	99.7
76	0.0403	1.80485	2.594	0.000	0.049	100.8
77	0.1008	1.80485	0.891	1.762	0.032	100.5
78	0.2016	1.80845	0.822	1.860	0.030	101.3
79	0.4033	1.80413	0.834	1.838	0.032	101.2
80	1.0082	1.80341	0.862	1.781	0.028	100.0

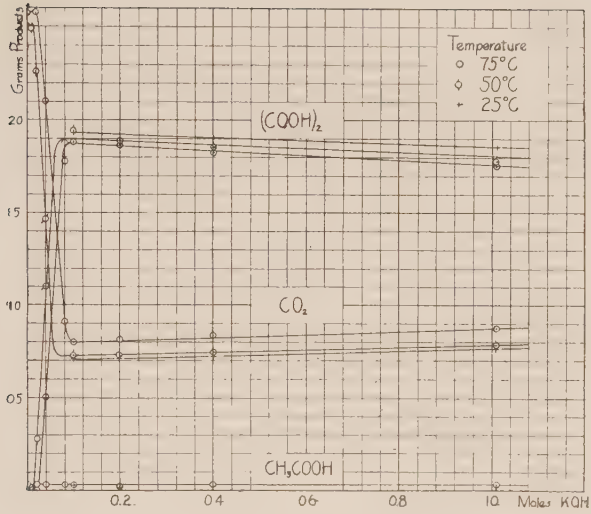


FIG 1 EFFECT OF TEMPERATURE ON OXIDATION OF 0.005 MOLE LACTOSE

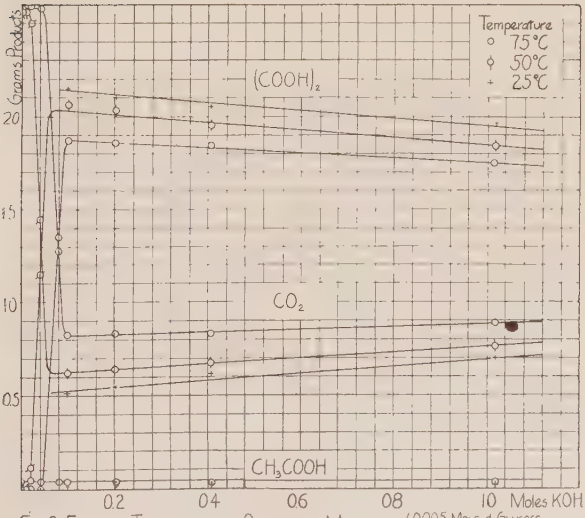


FIG 2. EFFECT OF TEMPERATURE ON OXIDATION OF MIXTURE OF { 0.005 MOLE d-GLUCOSE / 0.005 MOLE d-GALACTOSE

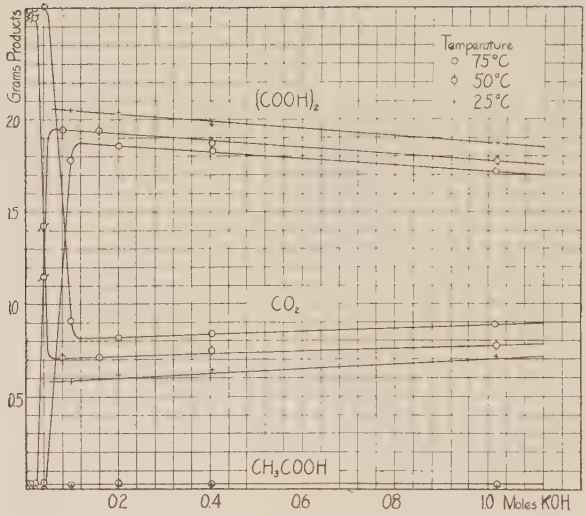


FIG. 3 EFFECT OF TEMPERATURE ON OXIDATION OF 0.01 MOLE d-GLUCOSE

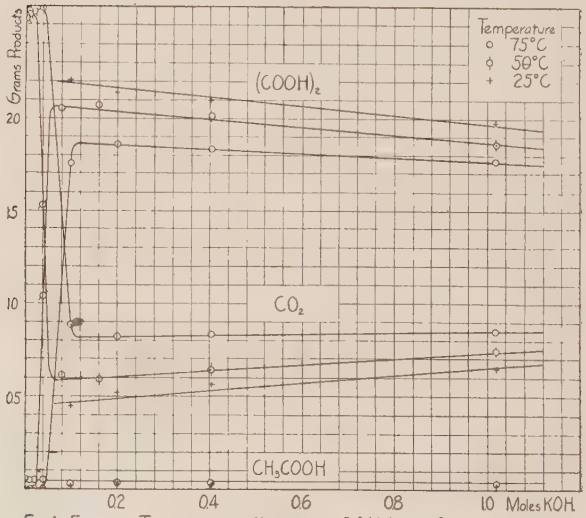


FIG 4. EFFECT OF TEMPERATURE ON OXIDATION OF 0.01 MOLE d-GALACTOSE

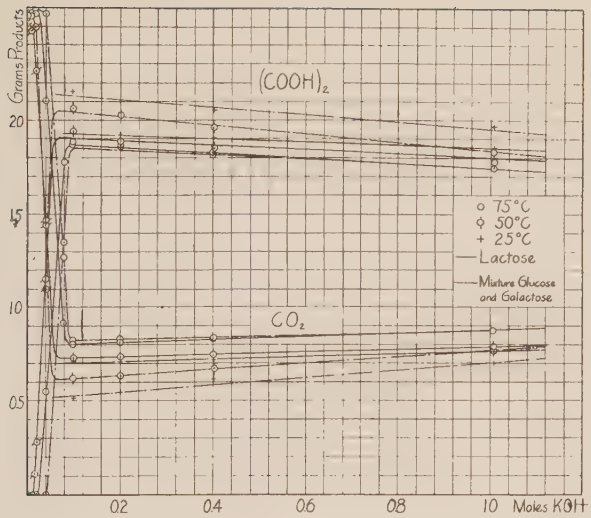


FIG. 5. COMPARISON OF AMOUNTS OF CO_2 AND $(COOH)_2$ FROM 0.005 MOLE LACTOSE AND MIXTURE OF 0.005 MOLE d-GLUCOSE AND 0.005 MOLE d-GALACTOSE

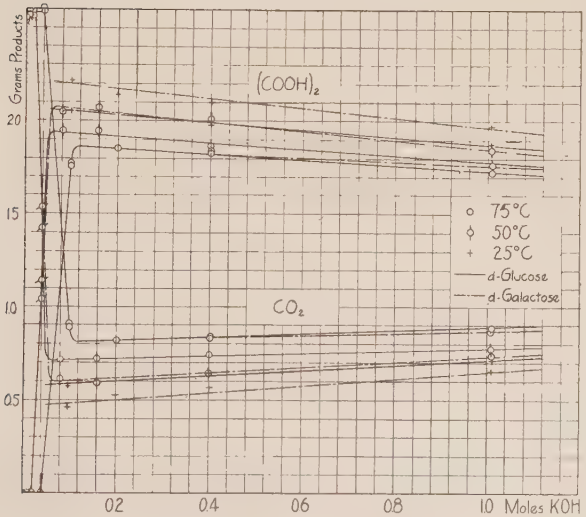


FIG. 6. COMPARISON OF AMOUNTS OF CO_2 AND $(COOH)_2$ FROM 0.01 MOLE d-GLUCOSE AND 0.01 MOLE d-GALACTOSE

DISCUSSION

The results as recorded in the tables show that the amounts of the sugars recovered as oxidation products vary from 97.4 to 102.0 per cent. Practically all the lower results were obtained in the case of the oxidations at 50° and since the first experiments were carried out at this temperature, these low yields were probably due to experimental errors. The variation of yields from 100 per cent can be tolerated when we consider not only that each determination had to be multiplied by at least 10 and in some cases 20, but that the determination for carbon dioxide is not considered accurate to nearer than 0.0010 gram.

Quite a few of the analyses on acetic acid are missing in the case of the oxidations at 50°. These results were slightly too high due to the failure to remove carbon dioxide from the phosphoric acid distillate before titrating against the standard alkali and therefore were omitted. Fortunately the amount of acetic acid was practically constant in all cases. Therefore, these discrepancies in no way interfered with any conclusions which might be drawn from these results.

(a) OXIDATION PRODUCTS.

(1) Carbon Dioxide.

Carbon dioxide was formed in all the oxidations. About 98% of the sugars oxidized to carbon dioxide at neutral but this amount decreased very rapidly upon the addition of alkali until a concentration of approximately 0.08N was reached when the amount of carbon dioxide began to gradually increase.

(2) Oxalic Acid.

Oxalic acid was not a final product in the oxidations at neutral. However upon the addition of alkali, it soon appeared and its curve ran just the opposite to that of carbon dioxide.

That oxalic acid is an intermediate product at neutral was shown as follows: An oxidation of d-glucose was carried out at room temperature (about 27°) in the manner described above. That no

oxalic acid is obtained after complete oxidation under these conditions was shown in the case of a ten day oxidation of d-glucose at 25°, which gave no oxalic acid. Since the carbon return was too low in this oxidation, it is not included in the data. The agitation of the run at room temperature was continued for 3 hours, after which the sodium hypophosphite was added and the mixture was filtered. The filtrate was treated in the usual manner with acetic acid and calcium acetate, and the resulting precipitate was filtered off and carefully washed with hot water. The precipitate was then dissolved in a small amount of hydrochloric acid and the solution was diluted to about 700 cc. A small amount of a 5% solution of calcium acetate was next introduced after which the mixture was heated to boiling. Ammonium hydroxide was now added very gradually during the boiling until the solution became slightly alkaline, after which the precipitate which had formed was allowed to settle. This precipitate after being filtered and washed until free from chlorides was dried for 3 hours at 105°. On converting this calcium salt to calcium sulfate by moistening it in a platinum crucible with sulfuric acid and then gradually heating, the following results were obtained:

Wt. of sample $CaC_2O_4.H_2O$ G.	Wt. of $CaSO_4$ G.	Calculated for $CaSO_4$ G.
0.1626	0.1500	0.1515
0.1966	0.1818	0.1832

These results point to the possibility of potassium oxalate being unstable in the presence of potassium permanganate. Mr. C. W. Kreger, of this laboratory, and myself have shown this to be true to a certain extent by a series of experiments as follows:

Potassium oxalate which analyzed 100.00% was agitated in neutral and alkaline solutions with 13.0000 grams potassium permanganate in 500 cc. of water for 4 days. The results were as follows:

Normality of KOH	$K_2C_2O_4.H_2O$ at beginning G.	$K_2C_2O_4.H_2O$ at end G.	Per cent recovery
0.0000	5.5246	4.9946	90.4
0.0184	5.5246	5.2097	94.3
0.0369	5.5246	5.2925	95.8
0.1845	5.5246	5.3070	96.1

These results show that potassium oxalate is not completely stable[71] in the presence of potassium permanganate but gradually decomposes, the amount of such decomposition depending on the alkalinity, the time, and probably also on the concentration of the potassium permanganate, which is always high in this case. However the rate of its decomposition is not as high as that of the oxalate found in the incompleted oxidations. One hundred cubic centimeters of the filtrate from a neutral glucose oxidation which had been run 2 hours at 50° required 9.92 cc. of 0.1N potassium permanganate in the determination for the oxalic acid present, while the decomposition of potassium oxalate at neutral amounted to only 5.12 cc. for 100 cc. of the filtrate. Since the runs with potassium oxalate were agitated longer than is necessary to complete the oxidations at neutral at 50°, it is evident that the oxalic acid cannot wholly be present as the normal potassium oxalate. Therefore, it is quite likely that at least some of the oxalic acid is present as the acid oxalate. A preliminary experiment with equivalent amounts of potassium acid oxalate and potassium permanganate gave a clear solution after standing one day at room temperature.

(3) Acetic Acid.

The acetic acid which was found in all the oxidations never amounted to more than 3% of the total carbon introduced as sugar. The amount of acetic acid was practically constant for all the sugars at any one temperature but there seemed to be a slight increase in this amount with increase in temperature.

(b) HISTORICAL—INTERPRETATION OF THE MECHANISM OF CARBOHYDRATE OXIDATION.

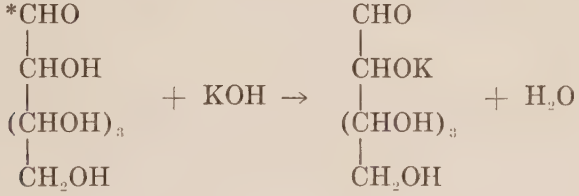
Although many investigators have oxidized the monosaccharides and disaccharides and recorded the products which they obtained, few have attempted to explain the mechanism by which they succeeded in getting these products. Nef[72] and his students[73] have

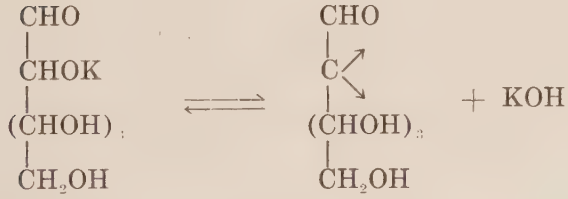
[71] Berthelot, Ann. Spl. **6**, 184, observed a slight decomposition after several days.
[72] Ann. **357**, 271.
Ann. **376**, 1.
Ann. **403**, 204.
[73] McLeod, *Am. Chem. J.* **37**, 25 (1907).
Anderson, *Am. Chem. J.* **42**, 401 (1909).
Spoehr, *Am. Chem. J.* **43**, 227 (1910).
Glattfeld, *Am. Chem. J.* **50**, 135 (1913).

advanced the most plausible explanation to account for the products of the oxidation of the hexoses in alkaline solution. Nef[74] thought that the alkali present formed a salt with the hexose, the metal replacing the hydrogen from one of the hydroxyl groups, and this salt formation therefore resulted in an increase in the amount of methylene dissociation due to the fact that the point of dissociation of the alcoholates thus formed is lower than that of the alcohol itself. In the case of ethyl alcohol, for example, the effect of alkali would be to increase the dissociation as follows:

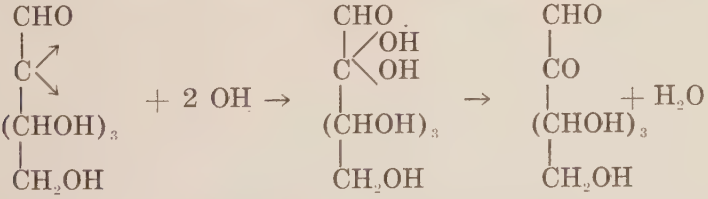
$$CH_3CH_2OH + KOH \leftrightarrows CH_3CH_2OK + H_2O \rightleftharpoons CH_3CH\langle + KOH + H_2O$$

With the hexoses, we would have the following dissociation:





This latter compound would then be oxidized as follows:



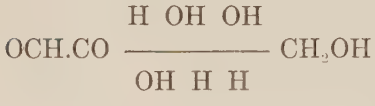
In the case of d-glucose, for example, we would then have the five

[74] Ann. **357**, 283.

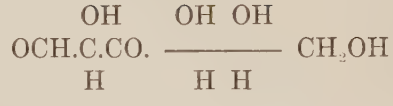
* The aldehyde formulas for the hexoses are used throughout this paper for the sake of simplicity.

following dicarbonyl compounds since there are five hydroxyl groups present in this hexose:

I



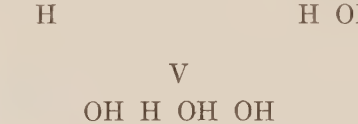
II



III

IV

V



The products of the oxidation resulted either from the direct oxidation of these osone compounds or from the splitting at the carbonyl group and subsequent oxidation.

Later Nef[75] assumed the intermediate formation of the 1:2, 2:3, and 3:4 dienols in the alkaline oxidation of the hexoses. Evidence for the existence of such compounds in the presence of alkalies was presented by McLeod[76] in the case of acetaldehyde which was shown to give vinyl alcohol as follows:

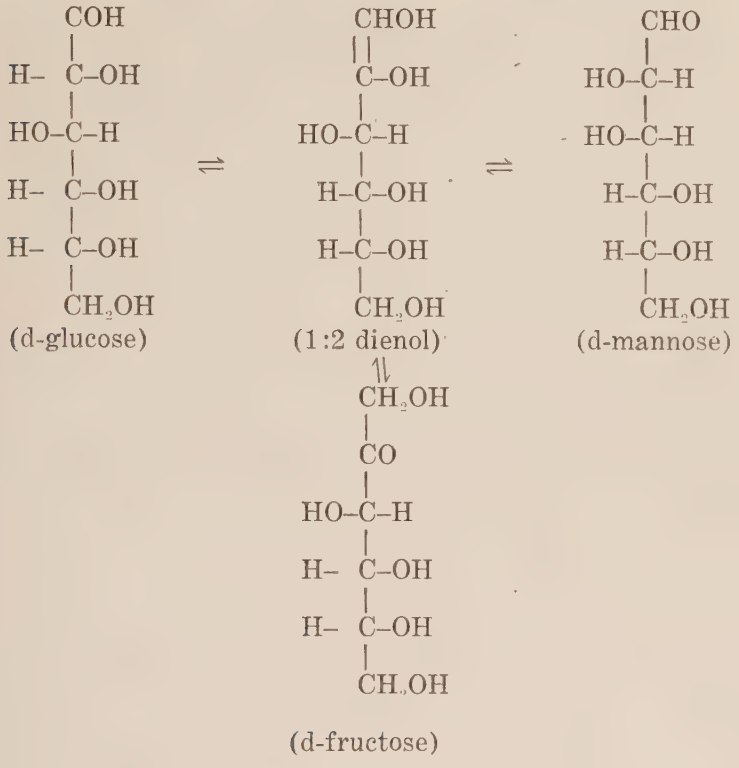
$$CH_3CHO \rightarrow CH_2C(OH)\langle \rightarrow CH_2{=}CH(OH)$$

Later, Evans and Looker[77] succeeded in showing by the formation of mercury derivatives that the amount of vinyl alcohol formed was proportional to the concentration of the alkali.

Furthermore, the discovery by Lobry de Bruyn and van Ekenstein[78] of the interconversion of glucose, fructose, and mannose in the presence of alkalies can best be explained on the assumption of

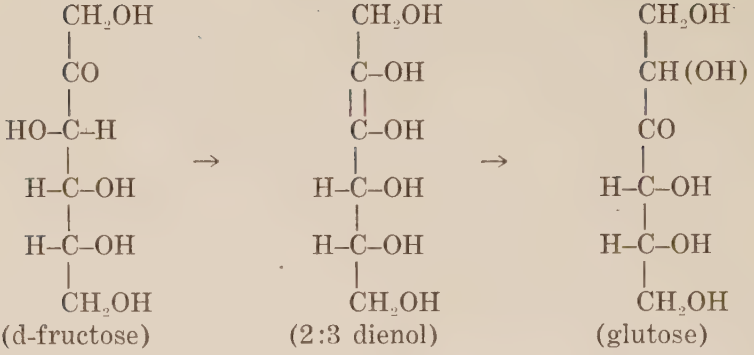
[75] Ann. **376**, 7; **403**, 205.
[76] *Am. Chem. J.* **37**, 25 (1907).
[77] *J. Am. Chem. Soc.* **43**, 1925 (1921).
[78] Rec. trav. chim. **14**, 203 (1895).

the intermediate formation of the 1:2 dienol. This equilibrium as first suggested by Wohl[79] may be represented as follows:



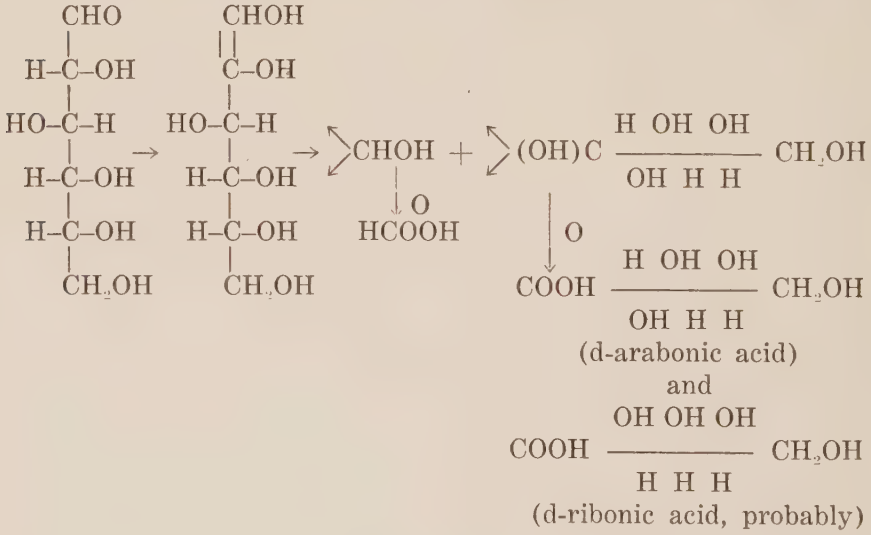
Later, Lobry de Bruyn[80] succeeded in showing that a similar equilibrium existed in the case of galactose but in addition other hexoses were isolated from the mixture. Further work on the glucose-fructose-mannose equilibrium likewise resulted in the additional discovery of pseudo-fructose and glutose, all of which are ketoses. Nef[81] concluded from theoretical considerations that glutose must be a mixture of two isomeric 3-ketohexoses. Its formation from fructose may be represented as follows:

[79] Armstrong's *"Simple Carbohydrates and Glucosides,"* Third Edition, pg. 45.
[80] Rec. trav. chim. **16**, 257, 262, and 274 (1897).
[81] Ann. **357**, 297.



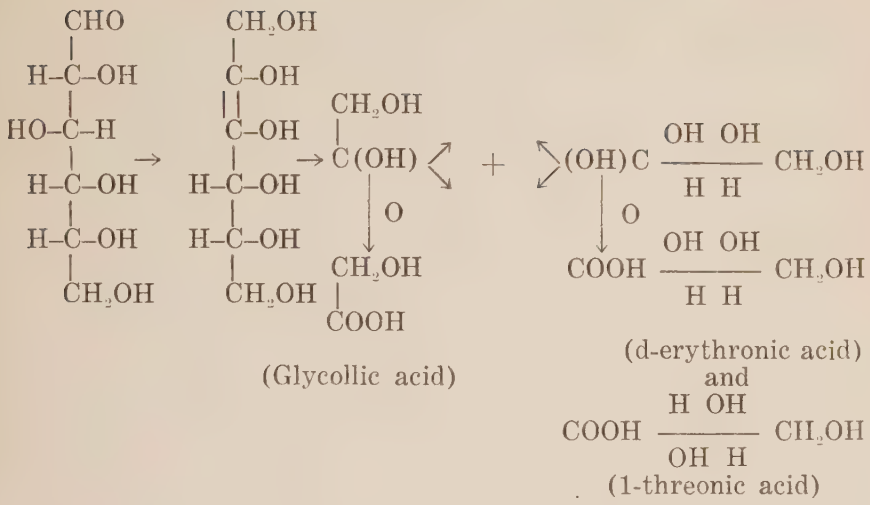
On these grounds, Glattfeld[82] has proposed the following explanation for the mild oxidation of d-glucose in alkaline solution:

(1) Formation of 1:2 dienol with subsequent splitting of the enol compound and the oxidation of the splitting products.

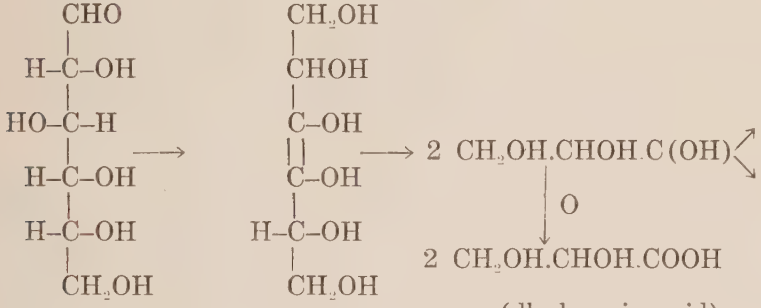


(2) Formation of 2:3 dienol and subsequent splitting and oxidation.

[82] loc. cit.



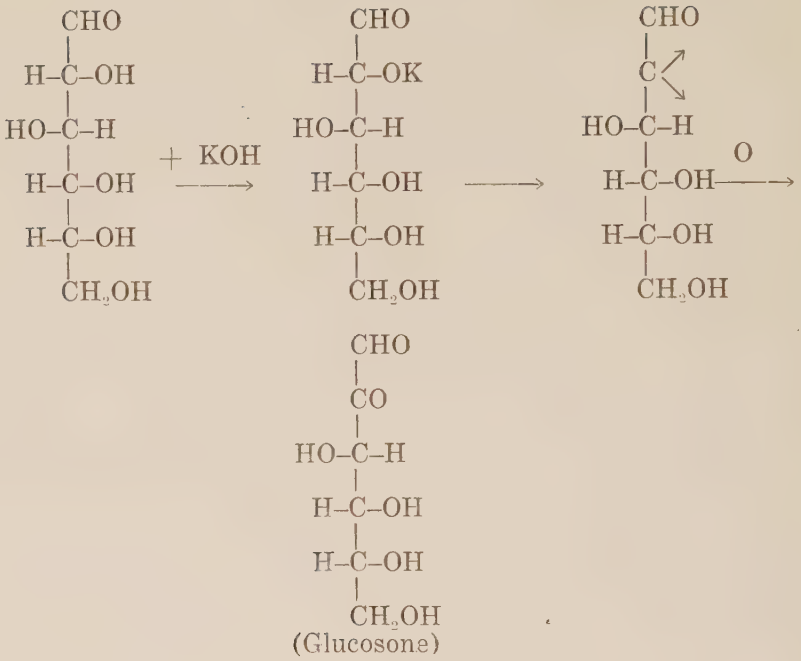
(3) Formation of 3:4 dienol and subsequent splitting and oxidation.



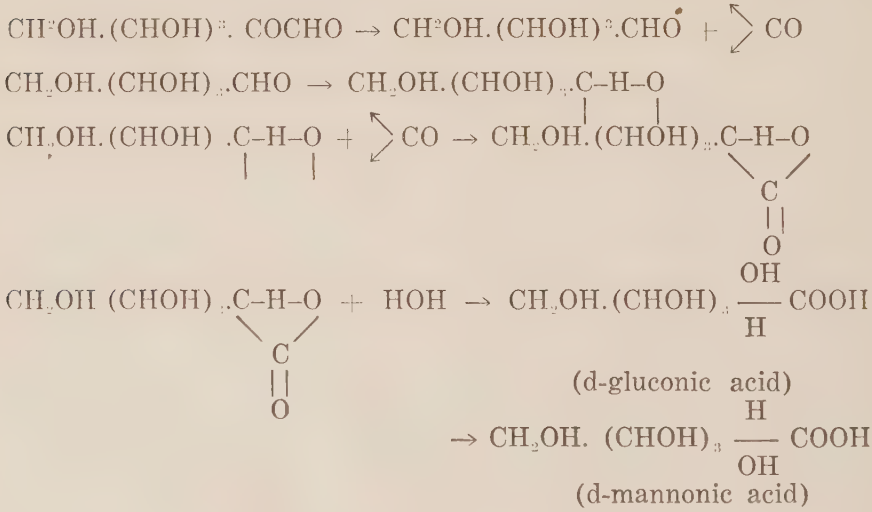
The above products, formic acid, d-arabonic acid and d-ribonic acid (probably), glycollic acid, d-erythronic and l-threonic acids, and dl-glyceric acid were all obtained by oxidizing glucose in alkaline solution by means of hydrogen peroxide.

Previous work by Nef[83] on the oxidation of glucose by Fehlings solution gave considerable quantities of hexonic acids although Glattfeld was unable to isolate any of these acids in his oxidation mixture. Nef has accounted for the formation of the hexonic acids as follows:

[83] Ann. **357**, 277.



The glucosone then undergoes the benzilic acid re-arrangement as follows[84]:



[84] Ann. **357**, 284.

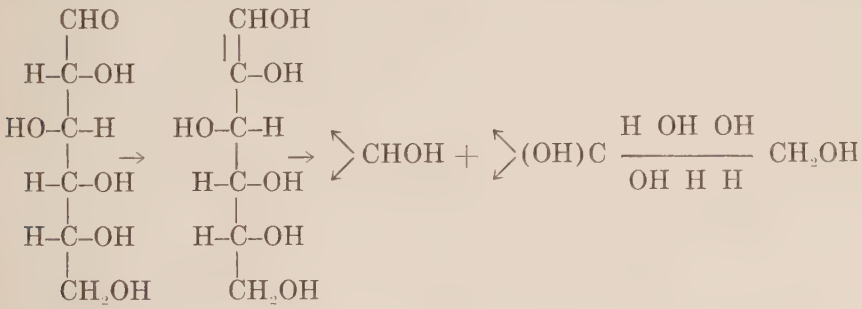
(c) VIEWS ON THE MECHANISM OF CARBOHYDRATE OXIDATION AS DRAWN FROM THE ABOVE DATA.

The oxidation of the carbohydrates, lactose, d-glucose, and d-galactose with the strong oxidizing agent, potassium permanganate, resulted in the formation of carbon dioxide, oxalic acid, and acetic acid. However it is logical to assume that the products found by Glattfeld, or rather the unoxidized enol forms of these products, were present as intermediate compounds in these oxidations, since in both cases alkaline solutions were used, the only difference being the oxidizing agents. It is very probable that in alkaline solutions the carbohydrates are all in the same state of dissociation, but the difference in the products of oxidation is simply due to the difference in the oxidizing potential of the oxidizing agent.

I—Effect of Variation in Concentration of Alkali on the Oxidation of d-Glucose.

Based on the theories of Nef it can then be postulated that d-glucose may undergo the dissociations as follows:

(1) The d-glucose may enolize forming the 1:2 dienol which then dissociates at the double bond forming hydroxymethylene and the methylenenol of d-arabinose.



That such a dissociation is possible follows from the fact that the 1:2 dienol most likely exists as an intermediate product in the interconversion of glucose, fructose, and mannose as discovered by Lobry de Bruyn and van Ekenstein.[85] If d-arabinose is formed in accordance with the dissociation represented in (1) we should expect its enolization into the 1:2 and 2:3 dienols and subsequent dissociation into hydroxymethylene, and the methylenenols of d-ery-

[85] loc. cit.

throse, glycollic aldehyde, and dl-glyceric aldehyde.[86] The d-erythrose would then probably enolize into its 1:2 and 2:3 dienols which would dissociate into hydroxymethylene, the methylenenol of dl-glyceric aldehyde and two molecules of the methylenenol of glycollic aldehyde. The total dissociation according to (1) may be represented as follows:

d-glucose → d-arabinose + formaldehyde
d-arabinose → d-erythrose + formaldehyde
d-arabinose → dl-glyceric aldehyde + glycollic aldehyde
d-erythrose → dl-glyceric aldehyde + formaldehyde
" → 2 molecules glycollic aldehyde

The dissociation products present after complete dissociation would be formaldehyde, glycollic aldehyde, and dl-glyceric aldehyde. In order that dissociation (1) may fit the facts, the oxidation products of these three compounds must agree with those obtained in these oxidations, i. e., at neutral, carbon dioxide and acetic acid only.

It is a well known fact that formaldehyde oxidizes to carbon dioxide and water with an excess of a strong oxidizing agent. The work of Evans and Adkins[87] on the oxidation of glycollic aldehyde and glycollic acid indicates that both these compounds would be oxidized to carbon dioxide alone at neutral with potassium permanganate. The lowest alkalinity which they used in the case of glycollic aldehyde was 1.2 grams potassium hydroxide per liter and at this point oxalic acid was still formed although the projected curve shows the absence of oxalic acid at zero alkalinity. These investigators obtained oxalic acid with potassium glycollate at neutral but the oxalic acid curve is dropping so abruptly at this point that it seems quite likely that they would have obtained carbon dioxide only had they used glycollic acid itself.

Likewise the oxidation of glyceric aldehyde[88] at neutral would undoubtedly yield carbon dioxide and acetic acid. From the above it is evident that carbon dioxide (in large amounts) and acetic acid (in small amounts) are the final products of the neutral oxidation of the intermediate compounds postulated. Therefore, the oxidation of d-glucose in neutral solution can be satisfactorily explained by dissociation (1).

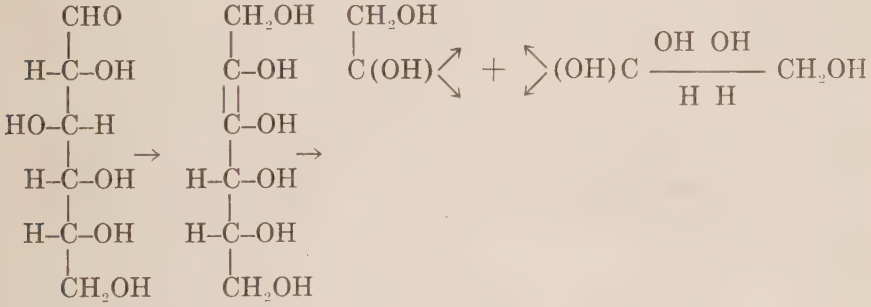
When potassium permanganate solutions are made alkaline by the addition of increasing amounts of alkali, the formation of oxalic

[86] Nef, Ann. **357**, 251, obtained the acids of these products in oxidizing arabinose.
[87] loc. cit.
[88] "from a private communication."

acid begins and the amount of carbon dioxide, obviously, begins to fall since the acetic acid remains constant. Evans and Adkins[89] have shown that glycollic aldehyde, as well as glycollic acid, is oxidized with alkaline permanganate to give oxalic acid as one of the reaction products. Furthermore, oxalic acid has been obtained in the alkaline oxidation of glyceric aldehyde.[90]

Qualitatively, therefore, dissociation (1) fits the facts in alkaline solution but on investigating quantitatively it is evident that the amount of oxalic acid produced according to this dissociation would be less than that actually obtained. According to Evans and Adkins,[91] 76% of the carbon in glycollic acid oxidizes to oxalic acid at 50° in the case of the higher alkalinities. d-Glucose at the same temperature and at an alkalinity of 0.0808N (Experiment 49) shows an oxalic acid yield of 72%. Therefore glycollic acid[92] is the principal intermediate compound, with the possible exception of glyceric acid,[93] since it is the only intermediate compound that produces as much oxalic acid as d-glucose. However, since it is not possible according to dissociation (1) for more than two-thirds of the carbon in glucose to go to glycollic acid (the other two carbon atoms must go to formaldehyde and then to carbon dioxide) it is evident that dissociation (1) does not satisfactorily explain the oxidation of d-glucose at the higher alkalinities.

(2) The d-glucose may enolize forming the 2:3 dienol which then dissociates at the double bond forming the methylenenols of glycollic aldehyde and d-erythrose.



[89] loc. cit.
[90] "from a private communication."
[91] loc. cit.
[92] Evidently the enol form of glycollic aldehyde in oxidizing completely to glycollic acid under these conditions must behave differently than ordinary glycollic aldehyde.
[93] Glyceric acid has never been oxidized.

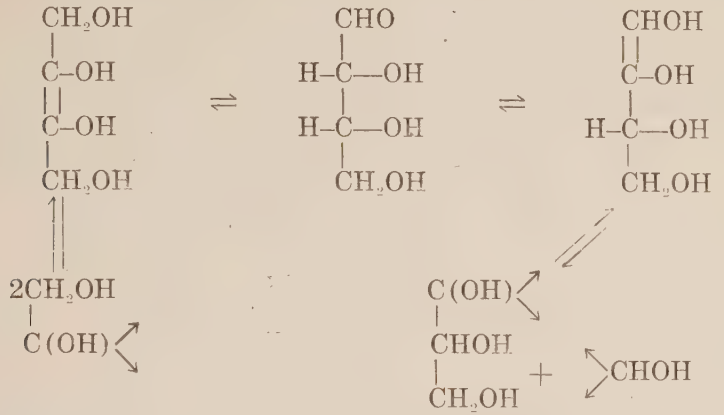
The fact that glutose and galtose[94] both of which have been shown to be 3-ketohexoses, have been obtained from glucose and galactose respectively, can only be explained on the intermediate formation of the 2:3 dienol. If d-erythrose is formed in accordance with (2) we should expect its enolization into the 1:2 and 2:3 dienols with subsequent dissociation into hydroxymethylene and the methylenenols of dl-glyceric and glycollic aldehydes. The total dissociation according to (2) may be represented as follows:

d-glucose → d-erythrose + glycollic aldehyde
d-erythrose → dl-glyceric aldehyde + formaldehyde
" → 2 molecules glycollic aldehyde

The dissociation products are therefore the same as in dissociation (1) which means that the oxidation of d-glucose at neutral can also be explained on the basis of dissociation (2). However at the higher alkalinities we have a different situation in that it is possible for d-glucose to dissociate into three molecules of the methylenenols of glycollic aldehyde. Evidence for such a dissociation is the fact that Nef[95] obtained large amounts of glycollic acid on oxidizing glucose with copper sulfate in alkaline solution. McLeod[96] and Spoehr[97] also obtained glycollic acid by the action of aqueous copper acetate and alkaline hydrogen peroxide, respectively, on glucose. On this assumption only can the high yields of oxalic acid be explained. As stated before the oxalic acid yield for glycollic acid at high alkalinities at 50° is 76% while the yield for d-glucose at 0.0808N is 72%. (Experiment 49). Evidently the d-glucose does not dissociate completely into three molecules of glycollic aldehyle methylenenol but a partial dissociation into dl-glyceric aldehyde methylenenol[98] and hydroxymethylene is also occurring.

[94] Lobry de Bruyn, loc. cit.
[95] Ann. **357**, 277.
[96] loc. cit.
[97] loc. cit.
[98] Mr. C. W. Holl, of this laboratory, has shown that the highest oxalic acid yield from glyceric aldehyde at 50° is 59.6%.

The equilibrium between d-erythrose and its dissociation products may be represented as follows:



The highest yield of oxalic acid in the oxidation of d-glucose at 50° is at 0.0808N alkali where the return is 72% (See Fig. 3). With increase in alkali the amount of oxalic acid gradually falls. The effect of alkali over 0.0808N is therefore to shift the above equilibrium to the right, since this shift results in the formation of more glyceric aldehyde and consequently less oxalic acid. Increase in temperature has the same effect only in a more pronounced way.

The fact that oxalic acid exists as an intermediate compound at low alkalinities may also be explained on the assumption that glycollic acid is the principal intermediate compound in these oxidations. Evans and Adkins[99] obtained oxalic acid at an alkali concentration of 0.23 grams per liter while in these oxidations none was obtained until an alkali concentration of 1.20 grams per liter was reached. This discrepancy is to be expected since it was necessary to agitate these oxidations much longer than the oxidations with glycollic acid and therefore the oxalic acid which we obtained as an intermediate compound was decomposed as has been shown in a special experiment above.

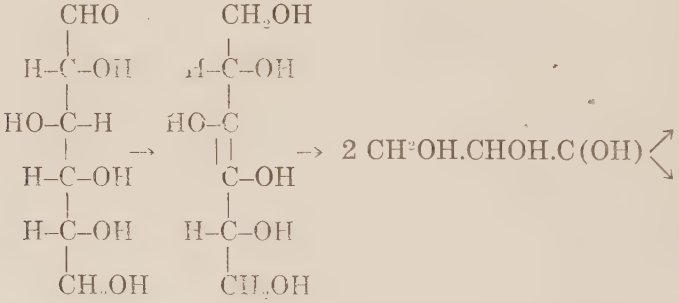
Therefore, the dissociation according to (2) explains the condition at neutral as well as at the higher alkalinities with the possible exception of acetic acid formation. The dissociation into glycollic aldehyde and consequent formation of glycollic acid may take place in either case, but the addition of alkali speeds up the reaction

[99] loc. cit.

to a great extent as is shown by the fact that the experiments at neutral and low alkalinities required more than six times as much time to go to completion as those at the higher alkalinities. Evidently the alkali increases the percentage of the 2:3 dienol present in a manner comparable to the formation of vinyl alcohol by increased amounts of alkali on acetaldehyde, as has been shown by Evans and Looker.[100] It may further hasten the dissociation of this compound into glycollic aldehyde methylenol and its effect on glycollic acid has been given by Evans and Adkins.[101] These investigators assumed the following dissociation:

Alkali shifts the above equilibrium to the right and thereby increases the amount of oxalic acid formed.

(3) The d-glucose may finally enolize forming the 3:4 dienol which would then dissociate into two molecules of glyceraldehyde methylenenol.



This enolization and dissociation results in but one product, glyceric aldehyde.[102] As is shown above the oxidation of glyceric aldehyde yields carbon dioxide and acetic acid at neutral. Dissociation (3) is therefore a possibility at neutral, but it is further evident from the above equations that the facts are not explicable at alkalinities over 0.0808N since at least one-third of the carbon must go to carbon dioxide.

[100] loc. cit.
[101] loc. cit.
[102] Nef and Glattfeld (loc. cit.) both obtained glyceric aldehyde in the oxidation of d-glucose.

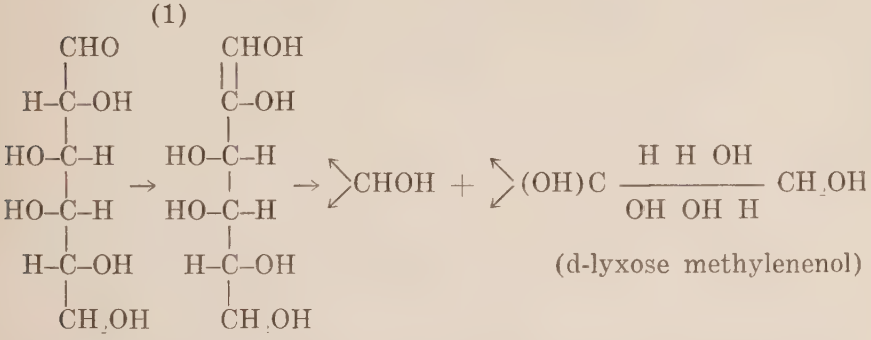
II—Effect of Variation in Concentration of Alkali on the Oxidation of d-Galactose.

The oxidation of d-galactose may be explained in a manner similar to that given for d-glucose. The essential difference is the fact that at alkalinities over 0.0808N at 25° and 50°, the yields of oxalic acid are higher in the case of d-galactose. This point is brought out very clearly by the curves on Fig. 6. Some of the actual experimental results are as follows:

Expt. No.	Normality of KOH	Sugar	Wt. of CO_2 G.	Wt. of $(COOH)_2$ G.
		Temperature 25°		
41	0.1010	d-Glucose	0.586	2.048
61	0.1010	d-Galactose	0.458	2.210
42	0.2020	d-Glucose	0.622	2.041
62	0.2020	d-Galactose	0.538	2.143
43	0.4040	d-Glucose	0.648	1.982
63	0.4040	d-Galactose	0.574	2.099
		Temperature 50°		
49	0.0808	d-Glucose	0.716	1.948
69	0.0808	d-Galactose	0.611	2.052
51	0.4044	d-Glucose	0.748	1.868
71	0.4044	d-Galactose	0.645	2.009

The following dissociations are possible for d-galactose:

(1)

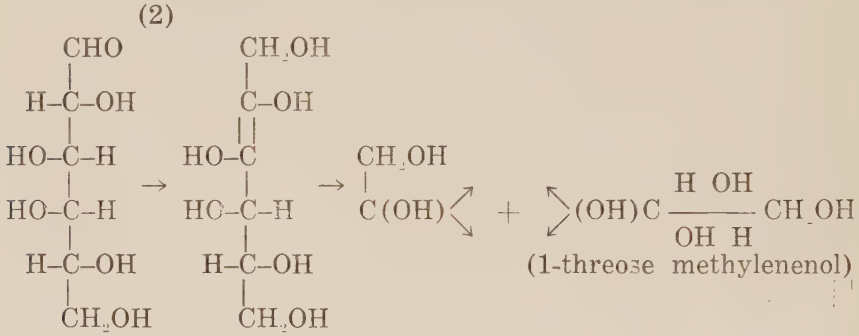


(d-lyxose methylenenol)

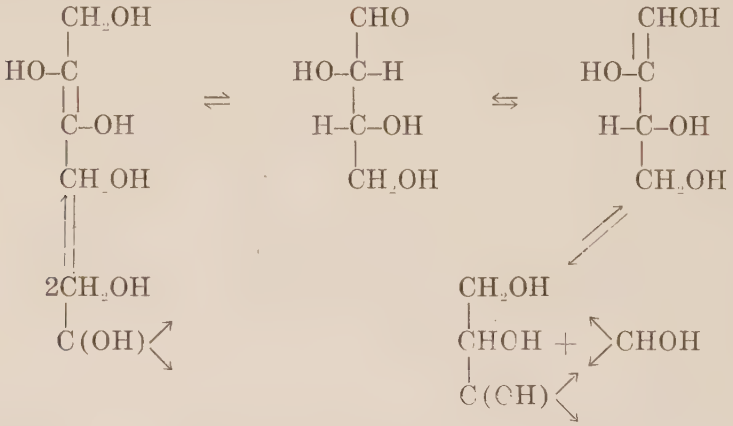
Here the d-lyxose methylenenol formed may dissociate into hydroxyformaldehyde and the methylenenol of l-threose or into the methylenenols of glycollic and dl-glyceric aldehydes. The l-threose methylenenol may further dissociate into hydroxyformaldehyde and the methylenenol of dl-glyceric aldehyde or into two molecules of glycollic aldehyde methylenenol. We then have the same possible intermediate compounds as with dissociation (1) under d-glucose and as in that case the oxidation of d-galactose at neutral may be explained according to (1), but the results at higher alka-

linities show a greater yield of oxalic acid than is available according to this dissociation.

(2)

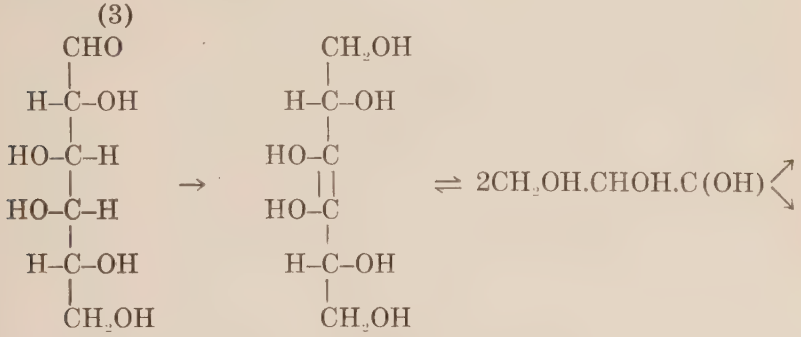


The l-threose methylenenol may further dissociate into hydroxyformaldehyde and dl-glyceric aldehyde methylenenol or into two molecules of the methylenenols of glycollic aldehyde. This latter dissociation may be represented as follows:



Dissociation (2) explains the oxidation at neutral and in addition the high yields of oxalic acid obtained from d-galactose at the higher alkalinities are explicable. Evidently the difference in oxalic acid formation from d-glucose and d-galactose must be attributed to the formation of two different tetroses, d-erythrose and l-threose, respectively. Under the same conditions of alkalinity and at temperatures of 25 and 50° a greater percentage of l-threose must dissociate to the left as compared to d-erythrose. The oxalic acid yield at 0.0808N for d-galactose at 50° is 76% (Experiment 69) while that for d-glucose under like conditions is 72% (Experiment 49)

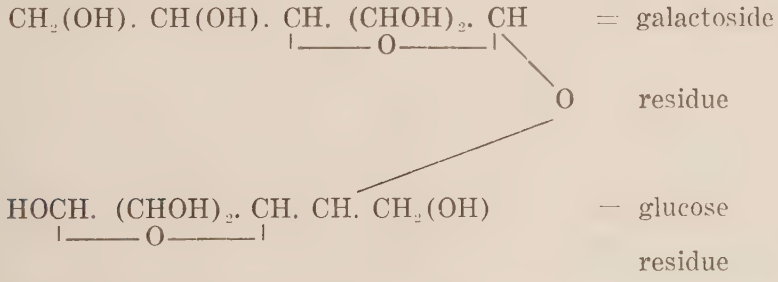
Since the yield obtained from glycollic acid, (76%), at the higher alkalinities is practically identical with that for d-galactose at 0.0808N, it follows that under these conditions l-threose must dissociate completely to the left. At 75° since the amounts of the products are the same for d-glucose and d-galactose, (See Tables IX and XII and Fig. 6), the percentages of dissociation are the same for d-erythrose and l-threose. The effect of temperature and the presence of oxalic acid as an intermediate compound may be explained as under d-glucose.



As under d-glucose this dissociation results in two molecules of dl-glyceric aldehyde methylenenol and can be used to explain the results at neutral.

III—Effect of Variation in Concentration of Alkali on Lactose and an Equivalent Mixture of d-Glucose and d-Galactose.

Lactose is a glucose β galactoside and its structural formula is as follows:



If lactose hydrolyzes into a mixture of glucose and galactose in the presence of alkalies we should expect the oxidation of this disaccharide to give the same amounts of oxidation products as an

equivalent mixture of its two hydrolytic splitting products. The results of these oxidations show that this is the case in but two instances: (a) at neutral at all the temperatures; and (b) in the case of alkalinities over 0.0807N at 75°. The curves on Fig. 5 as well as the actual results, which follow, show the agreement as mentioned under (b).

Expt. No.	Normality of KOH	Temperature 75° Sugar	Wt. of CO_2 G.	Wt. of $(COOH)_2$ G.
17	0.1008	Lactose	0.801	1.891
37	0.1008	Mixture Glucose and Galactose	0.822	1.871
18	0.2016	Lactose	0.816	1.878
38	0.2016	Mixture Glucose and Galactose	0.836	1.861
19	0.4033	Lactose	0.838	1.827
39	0.4033	Mixture Glucose and Galactose	0.834	1.851
20	1.0082	Lactose	0.885	1.750
40	1.0082	Mixture Glucose and Galactose	0.889	1.757

The condition at neutral is to be expected since it is quite likely that all the monosaccharides and disaccharides, when oxidized according to the conditions of these experiments, will produce acetic acid and carbon dioxide at neutral. However, the results at 75° with the higher alkalinities seem to indicate that under these conditions lactose splits into glucose and galactose before oxidation takes place. This is evidently due to the fact that at 75° above 0.0807N we have an "OH" concentration great enough to bring about this splitting of the lactose molecule into glucose and galactose.

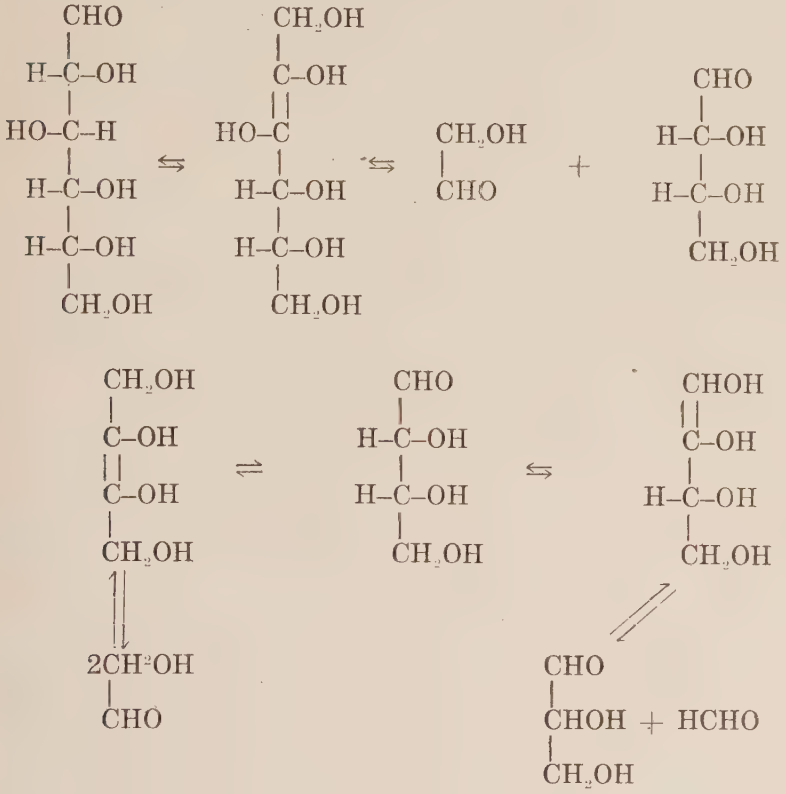
As is to be expected the results obtained for the mixture of the two hexoses agree very well for the average values for d-glucose and d-galactose. Obviously the effect of alkalies and temperature can be explained as with the individual hexoses given above. The oxidation of lactose at 75° in the case of the higher alkalinities can be explained in a similar manner. Due to the fact that little is known about the effect of alkalies on the complex lactose molecule, no attempt will be made to explain the mechanism of the oxidation of lactose at the other conditions followed in these experiments.

IV—Effect of Temperature.

The effect of temperature has been mentioned in the previous discussion but may be summarized here.

(1) d-Glucose.

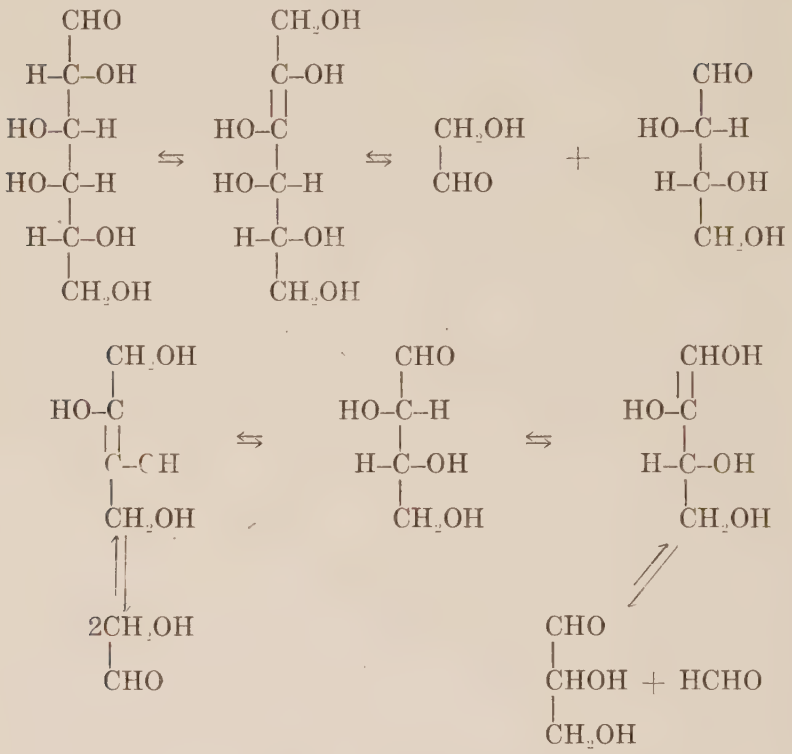
The principal dissociation in this case is:



The effect of temperature is to shift the equilibrium between d-erythrose and its dissociation products to the right, thus resulting in an increase in carbon dioxide production at the expense of the oxalic acid production.

(2) d-Galactose.

The principal dissociation in this case is:



At 50° with 0.0808N alkali, the dissociation of l-threose goes completely to the left. Increase in temperature increases the dissociation to the right, thereby decreasing the amount of oxalic acid and at the same time increasing the amount of carbon dioxide formed.

(3) Lactose and an Equivalent Mixture of d-Glucose and d-Galactose.

The effect of temperature on the mixture of d-glucose and d-galactose can be explained by a combination of (1) and (2). No attempt has been made to explain this effect on lactose, but it is to be noted from Fig. 5 that the amounts of products vary but little with the change in temperature.

SUMMARY

1. Lactose, an equivalent mixture of d-glucose and d-galactose, d-glucose, and d-galactose have been oxidized by means of potassium permanganate under varying conditions of temperature and concentration of potassium hydroxide. The products of these oxidations were carbon dioxide, oxalic acid, and acetic acid.

2. One of the uses of the potassium hydroxide is to neutralize the acids formed. In this way oxalic and acetic acids are stabilized.

3. The oxidation of d-glucose and d-galactose resulted in the same amounts of oxidation products except in the case of alkalinities over 0.0808N at 25° and 50°. In these cases the yield of oxalic acid was greater in the case of d-galactose.

4. Lactose gave different amounts of oxidation products as compared to the equivalent mixture of d-glucose and d-galactose except at alkalinities over 0.0807N in the case of 75 . It seems possible that lactose may, therefore, dissociate into glucose and galactose before oxidation under these conditions.

5. The amount of acetic acid was never greater than 3% of the total carbon introduced and it was practically constant for all the sugars at all the temperatures.

6. Oxalic acid was found to exist as an intermediate compound in the oxidations at neutral and low alkalinities.

7. Potassium oxalate is not perfectly stable in potassium permanganate solution. The amount of its decomposition depends on the strength of the alkali, the time, and probably on the concentration of the potassium permanganate.

8. The results obtained indicate that the hexoses dissociate

principally as follows under the conditions employed in these experiments:

(a) d-glucose $\leftrightharpoons$ d-erythrose + glycollic aldehyde

d-erythrose

2 mols glycollic aldehyde glyceric aldehyde + formaldehyde

(b) d-galactose $\leftrightharpoons$ l-threose + glycollic aldehyde

l-threose

2 mols glycollic aldehyde glyceric aldehyde + formaldehyde

9. The effect of alkali is as follows:

At neutral in 8(a) and 8(b) d-erythrose and l-threose dissociate largely into glycollic aldehyde which oxidizes to glycollic acid and then to carbon dioxide. As the alkali is increased oxalic acid begins to form due to the oxidation of glycollic acid and reaches a maximum at an alkali concentration of approximately .08N. With increase in alkali the oxalic acid curve begins to gradually drop due to the fact that under these conditions d-erythrose and l-threose dissociate to a greater extent to the right.

10. The difference in amounts of products obtained from d-glucose and d-galactose at 25° and 50° above 0.0808N alkali is attributed to the difference in the percentages of dissociation of d-erythrose and l-threose into glycollic aldehyde, and dl-glyceric aldehyde and formaldehyde.

11. The effect of temperature is also to shift the equilibria of d-erythrose and l-threose in 8 (a) and 8 (b) to the right, thereby causing an increase in carbon dioxide and a decrease in oxalic acid production.

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AUTOBIOGRAPHY

I, Calvin Adam Buehler, was born in Stone Creek, Ohio, November 29, 1896. My elementary education was received in the public school of that village; my secondary education, in the high school at Dover, Ohio; and my undergraduate education, at the Ohio State University, from which I obtained the degree of Bachelor of Chemical Engineering in 1918. During the following year I was employed as research chemist by The Barrett Company at Philadelphia, Pennsylvania; but in the Fall of 1919 I returned to the Ohio State University, where, in 1920, I received the degree of Master of Science. I was awarded the Du Pont Fellowship for the year 1921-22 and received the degree of Doctor of Philosophy in 1922.